DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

R & D STATUS REPORT

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$816,581.00

SHORT TITLE OF WORK

ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES

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QUARTERLY REPORT

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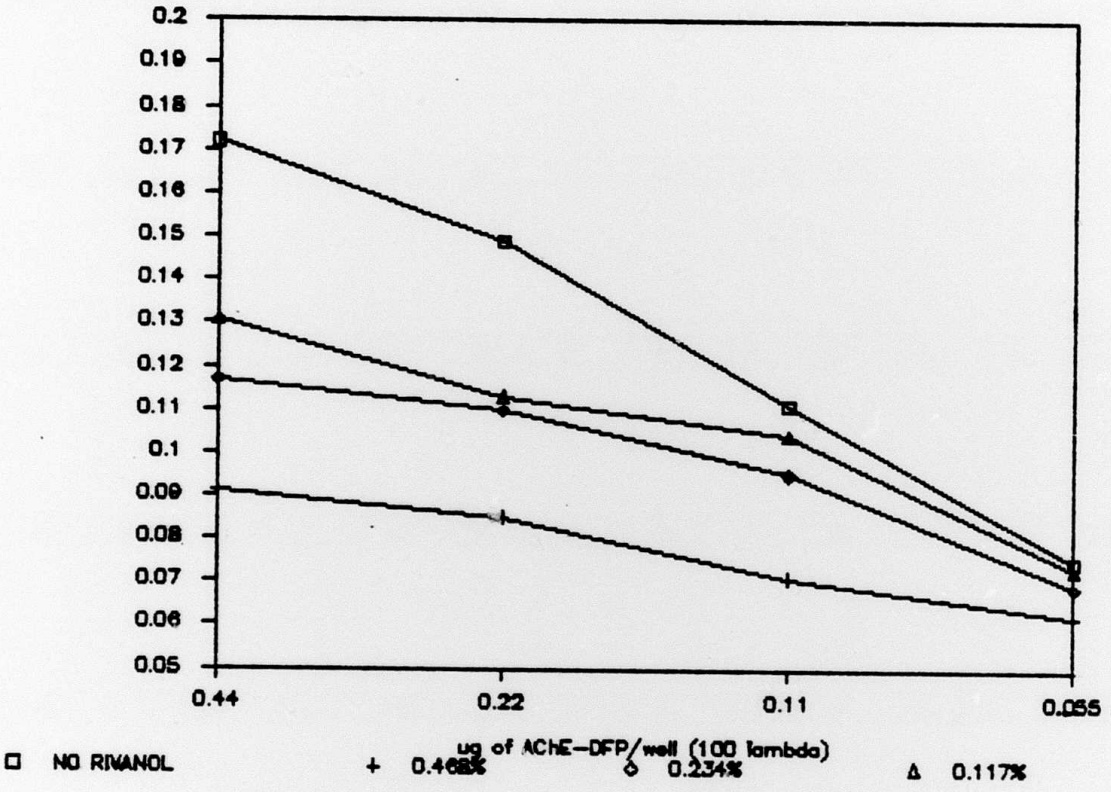
Vital to any tight binding detection system is the strength of the bond joining the signal to the target molecule. For antibody/ antigen systems, this requires a low dissociation constant and a correspondingly high intrinsic affinity constant. We have calculated an affinity constant for our AChE/DFP specific Ab_1 of $10^9 M^{-1}$. Because association constants are relatively uniform, being about $10^6 M^{-1}$ sec^{-1}, the dissociation constant for our antibody should give half lives of dissociation of about 1 to 10 seconds. We are encouraged by this result since it is at the high end of the range for haptens and leads us to believe that the antibody is recognizing the hapten AChE conjugate. In addition, this antibody represents one of our first isolated antibodies and we expect that antibodies of higher affinity can be found by screening greater numbers of monoclonal antibodies for higher affinity constants.

For the past year we have used passive binding to create a surface of AChE or another antigen on a polystyrene support. For an efficient ultrasensitive detection system, it is important that the trapping agent (AChE) can be bound to the solid support at high density. In the past months we have discovered that Rivanol, an agent used normally to purify antibody, significantly increases the binding of AChE and selected other proteins to polystyrene solid supports. While our investigation of the mechanism of how Rivanol increases binding is limited, the data does indicate increased binding which is pH dependent. Illustrated in the four graphs that follow is the pH and concentration effect of Rivanol on the binding of AChE/DFP conjugate.

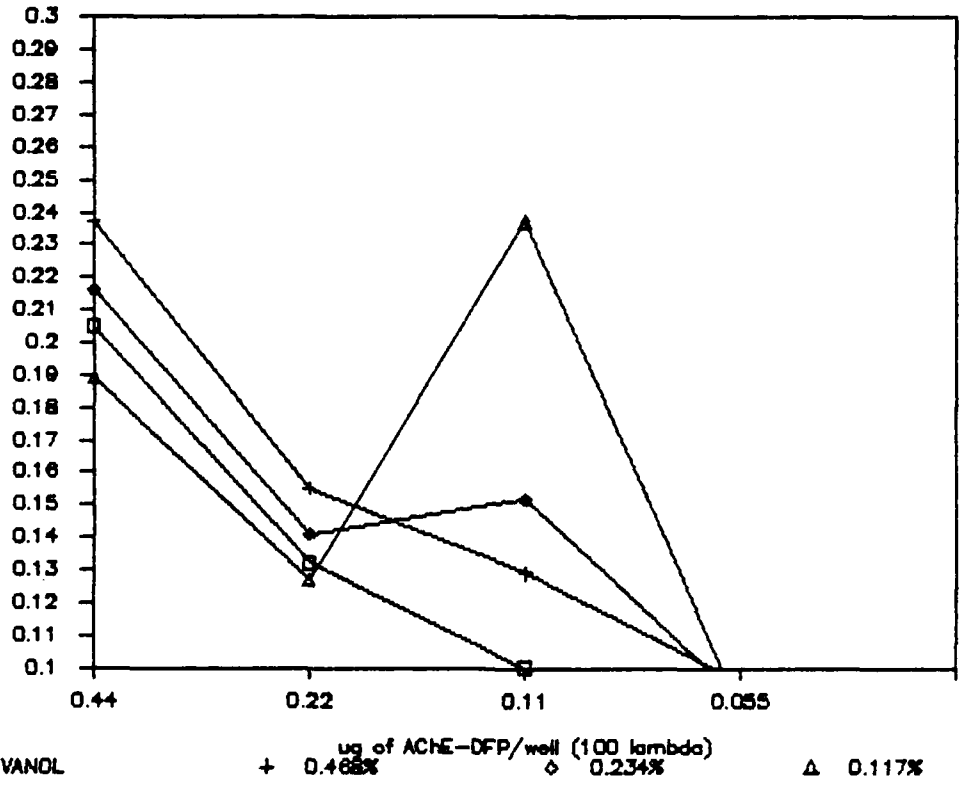
We have been working for some time to produce a platform for solid phase detection which is a flexible, easily handled system. We have recently produced a detection platform that is both a versatile experimental tool and could serve as the basis of a field testng device. This simple system mounts small plastic or polymer surfaces in a low volume well, adapted to treatment and testing. The advantages of this system over the polystyrene dishes previously used are many. The smaller reaction volume (200 lambda) saves valuable reagents and allows a 10 fold increase in the concentration of reactants (beads loaded with antibody). Increased concentration of reactants will provide much faster reaction kinetics between the antigen and antibody. Thus, our current reaction time in excess of 2 hours could be reduced to 10 minutes or less. In addition, the platform is adapted to ultracentrifugation for determining binding strengths. This system will give better washing because it allows the edges of the material being washed to be washed with the same efficiency as the central portions of the material. Lastly, this system permits better visualization and counting of fluorescent beads by reducing the interfering fluorescence of buffers and support materials. We believe this will allow us to more easily visualize smaller detector beads in the range of 0.3 micron diameter. Smaller beads should contribute to more stable antigen/antibody bonds by reducing bond stress and allow more rapid reaction kinetics by increasing interacting surface area. Thus we expect to decrease noise and increase specific reaction rates for testing a wide variety of surface polymers with a platform adaptable to field conditions.

pH 4

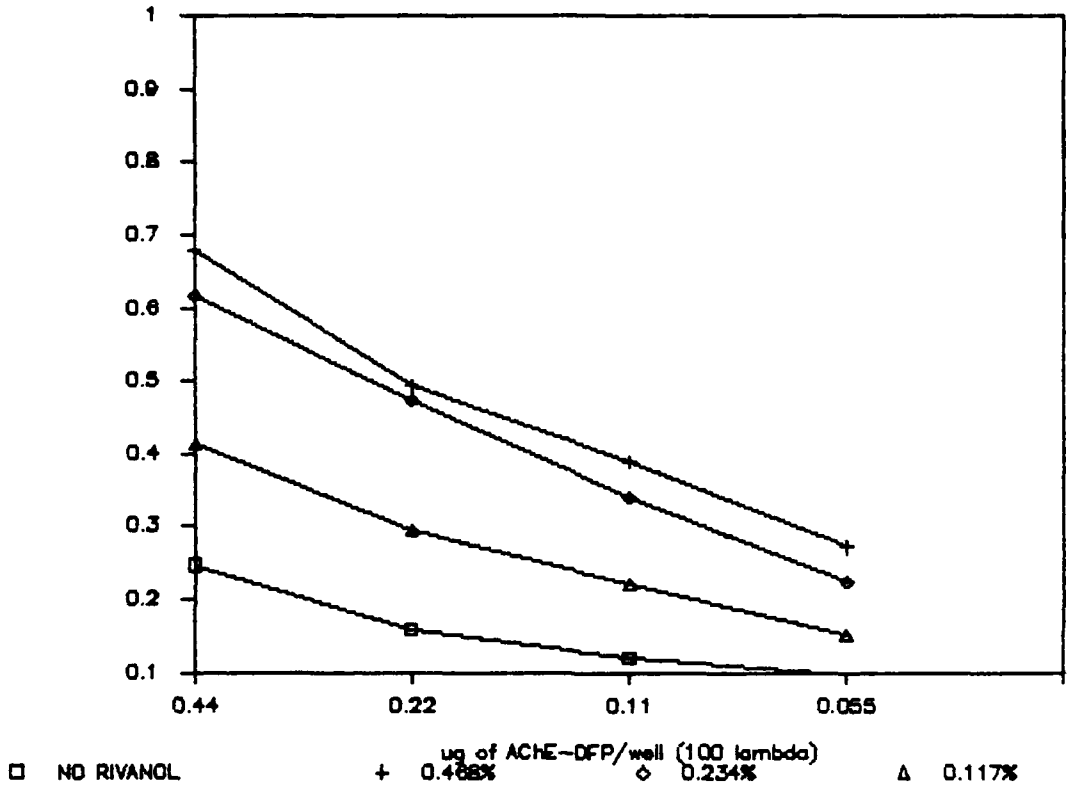


Percentages refer to percentage of 15 mM solution

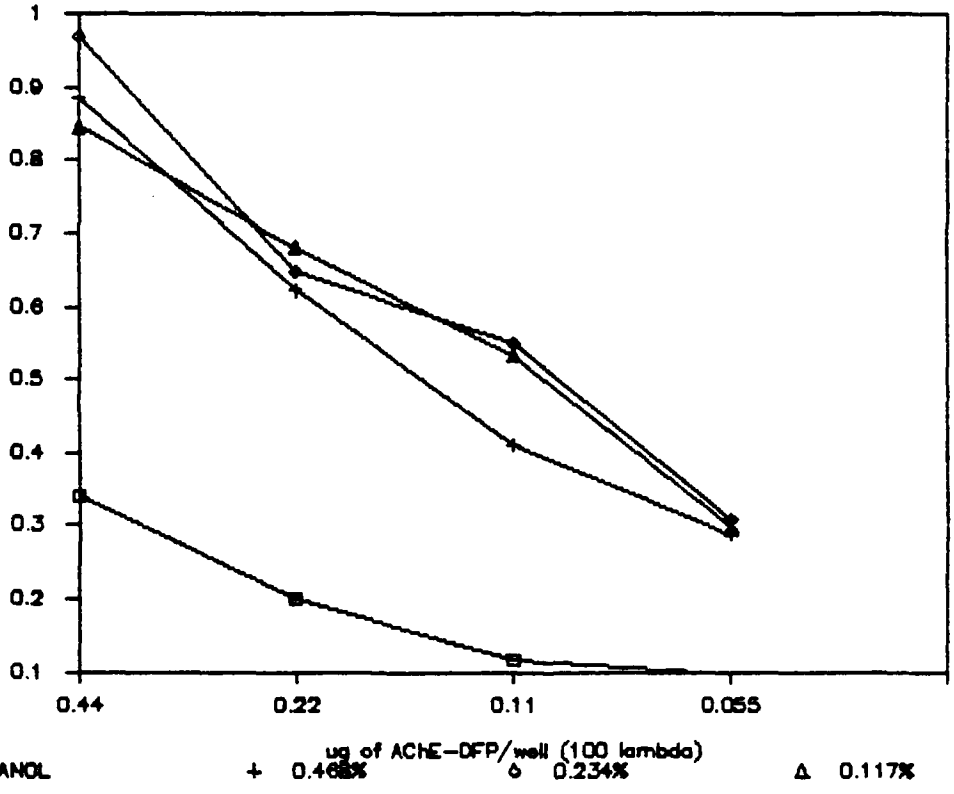
pH 6


0.3
0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.44
0.22
0.11
0.055
ug of AChE-DFP/well (100 lambda)
NO RIVANOL
0.468%
0.234%
0.117%

pH 8


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.44
0.22
0.11
0.055
ug of AChE-DFP/well (100 lambda)
NO RIVANOL
0.468%
0.234%
0.117%

pH 10


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.44
0.22
0.11
0.055
ug of AChE-DFP/well (100 lambda)
NO RIVANOL
0.468%
0.234%
0.117%

AChE/DFP at the levels indicated were plated in universal buffer at pH 4,6,8, and 10, containing either no Rivanol or .468%, .234%, or .117% of 15mM Rivanol, in a total well volume of 0.1 ml. The plates were incubated at 4'C on a rotating table for 16 hours, washed 3X with buffer containing .05% Tween 20. 0.1 ml of hybridoma SN was added to each well and incubated for 1 hour. The plates were washed 3X with buffer as above and 0.1 ml of 1:1000 anti-mouse IgG-HRP in buffer + Tween 20 was placed in each well. Plates were incubated at room temperature for 1 hour on a rotating table and washed 3X with buffer. Substrate buffer containing phenolenediamine and H_2O_2 was added and the OD_{405} of the wells after 30 minutes was determined.

It is clear that the amount of Rivanol necessary to increase binding is small. Binding is increased 2 to 3 fold at concentrations (4.4 ugm/ml) which shold be close to saturation of the surface with protein. In other experiments binding has been increased as much as 10 fold and enzymes appear to remain active. This system of binding may offer an attractive alternative to covalent methods of binding antigens at high densities.

Noise levels are critical to our ultrasensitive assay and we have completed work to reduce noise levels on our polystyrene supports. With our standard assay, the source of most of our non-specific binding (about 2 X 10^3 beads per $10mm^2$) is an

interaction between the solid matrix (polystyrene) of the bead and surface. This has been described in a previous quarterly report. In separate experiments designed to control this type of noise, we have reduced the non-specific binding of untreated beads to untreated surface by a factor of 10^3 through the use of buffers with lowered ionic strength. Thus our major source of noise is reducable by a factor of 10^3. Using buffers of reduced ionic strength in our test system should easily reduce the non-specific noise significantly - under 1,000 beads on our test surface. If a factor of 10^3 reduction in noise can be achieved with appropriate buffers, then the noise problem may be eliminated for all practical purposes. We have also tested polymer surfaces with lower noise characteristics than polystyrene.